



Antifungal Susceptibility Testing


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Why AFST?

- changing epidemiology of isolated agents
- increased of drug resistance
- newer drugs-more choice
- drug discovery
- optimizing therapy
- predict patients outcome

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- No standard therapy, unfavourable results, very limited data
 - Evaluation of new generation of triazoles and echinocandins
 - Sufficient data on in vitro activity can be performed to improve the managements of infections

Antifungal Susceptibility Testing

- Broth-Based Methodology: MIC
(Minimal inhibitory concentration).

CLSI (ex NCCLS) guidelines.

- Colorimetric methods: MTT, XTT, sensititre.
 - E-test determination

Methods for susceptibility testing

Broth dilution

- Macrodilution and Microdilution





Clinical and Laboratory Standards Institute (CLSI)

For yeasts

- M27-P: 1992
- M27-T: 1995
- M27-A: 1997
- M27-A2: 2003
- M44-P: 2004
- M27-A3: 2008
- M60: 2018

For filamentous fungi

- M38-P: 1998
- M38-A: 2003
- M38-A2: 2008
- M61: 2018

Drugs

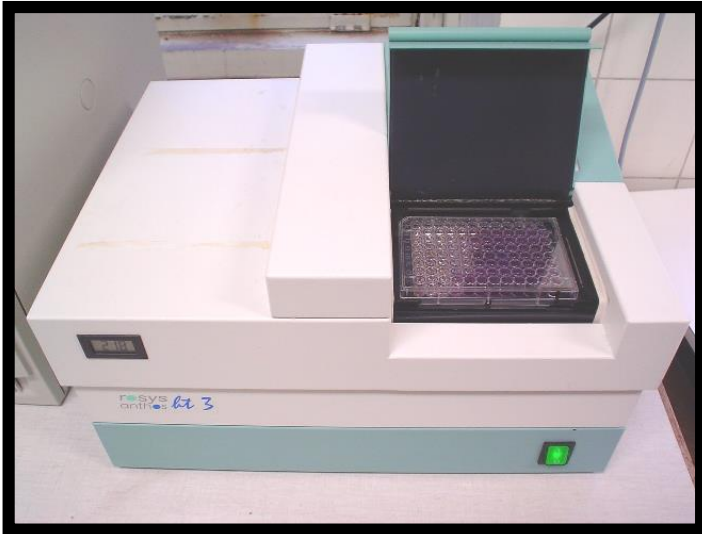


- AMB: amphotericin B.
- KTZ: ketoconazole.
- ITZ: itraconazole.
- FCZ: fluconazole.
- VCZ: voriconazole.
- PCZ: posaconazole.
- TBF: terbinafine.
- 5-FC: flucytosine.
- Equinocandins

Visual reading



Spectrophotometric reading



MIC bepaling m.b.v. batch maart 2003

Measurement file : 521.dat No error detected Measurement date : 4-4-03
 Template file : Plaatjes Schimmels 405 nm maart 2003.par Measurement time : 10:21:22

		Naam / Samples / OD Correctie / %Groei / MIC											
		1	2	3	4	5	6	7	8	9	10	11	12
A	Fluco	Fluco	Fluco	Fluco	Fluco	Fluco	Fluco	Fluco	Fluco	Fluco	Fluco	Fluco	Groei Con
	64,000	32,000	16,000	8,000	4,000	2,000	1,000	0,500	0,250	0,125	0,063	0,000	0,195
	0,179	0,153	0,181	0,164	0,177	0,166	0,185	0,166	0,181	0,162	0,188	0,195	100,0
	91,8	83,6	92,8	94,4	90,8	85,1	96,4	95,4	92,8	83,1	96,4	4	4
	4	4	4	4	4	4	4	4	4	4	4	4	4
B	5-FC	5-FC	5-FC	5-FC	5-FC	5-FC	5-FC	5-FC	5-FC	5-FC	5-FC	5-FC	Groei Con
	64,000	32,000	16,000	8,000	4,000	2,000	1,000	0,500	0,250	0,125	0,063	0,000	0,203
	0,057	0,056	0,076	0,112	0,153	0,192	0,238	0,306	0,189	0,193	0,190	0,190	100,0
	28,1	32,5	37,4	55,2	75,4	94,8	117,2	150,7	93,1	95,1	93,6	4	4
	3	3	3	4	4	4	4	4	4	4	4	4	4
C	Ampho B	Ampho B	Ampho B	Ampho B	Ampho B	Ampho B	Ampho B	Ampho B	Ampho B	Ampho B	Ampho B	Ampho B	Groei Con
	16,000	8,000	4,000	2,000	1,000	0,500	0,250	0,125	0,063	0,031	0,016	0,000	0,770
	-0,170	-0,128	0,029	-0,025	-0,013	-0,015	-0,009	-0,008	0,211	0,212	0,168	0,168	100,0
	-22,1	-16,8	-3,8	-3,2	-1,7	-1,9	-1,2	-1,0	27,4	27,5	24,4	4	4
	0 MIC	0 MIC	0 MIC	0 MIC	0 MIC	0 MIC	0 MIC	0 MIC	4	4	4	4	4
D	Itra	Itra	Itra	Itra	Itra	Itra	Itra	Itra	Itra	Itra	Itra	Itra	Groei Con
	16,000	8,000	4,000	2,000	1,000	0,500	0,250	0,125	0,063	0,031	0,016	0,000	0,222
	-0,009	-0,011	0,001	0,004	0,003	0,003	0,004	0,114	0,174	0,190	0,194	0,194	100,0
	-4,1	-5,0	0,5	1,8	1,4	1,4	1,8	51,4	76,4	85,6	87,4	4	4
	0 MIC	0 MIC	1 MIC	1 MIC	1 MIC	1 MIC	1 MIC	3	4	4	4	4	4
E	Verico	Verico	Verico	Verico	Verico	Verico	Verico	Verico	Verico	Verico	Verico	Verico	Groei Con
	16,000	8,000	4,000	2,000	1,000	0,500	0,250	0,125	0,063	0,031	0,016	0,000	0,221
	0,004	0,007	-0,024	0,005	0,008	0,030	0,764	0,174	0,193	0,210	0,199	0,199	100,0
	1,8	3,2	-10,9	2,7	3,6	13,8	345,7	78,7	87,3	85,0	90,0	90,0	4
	1 MIC	1 MIC	0 MIC	1 MIC	1 MIC	1 MIC	4	4	4	4	4	4	4
F	Terb	Terb	Terb	Terb	Terb	Terb	Terb	Terb	Terb	Terb	Terb	Terb	Groei Con
	1,000	0,500	0,250	0,125	0,063	0,031	0,016	0,008	0,004	0,002	9,77E-4	0,000	0,222
	0,000	0,000	0,002	-0,001	0,001	0,001	0,044	0,076	0,251	0,135	0,161	0,161	100,0
	0,0	0,0	0,9	-0,5	0,5	0,5	19,8	34,2	113,1	60,8	72,5	72,5	4
	1 MIC	1 MIC	1 MIC	0 MIC	1 MIC	1 MIC	1 MIC	4	4	3	4	4	4

12: growth control

11-1: dilutions of the drug

- Colorimetric methods: MTT, XTT, sensititre

Courtesy of
The Geraldine Kaminski Medical Mycology Library
Produced by: David Ellis and Roland Hermanis
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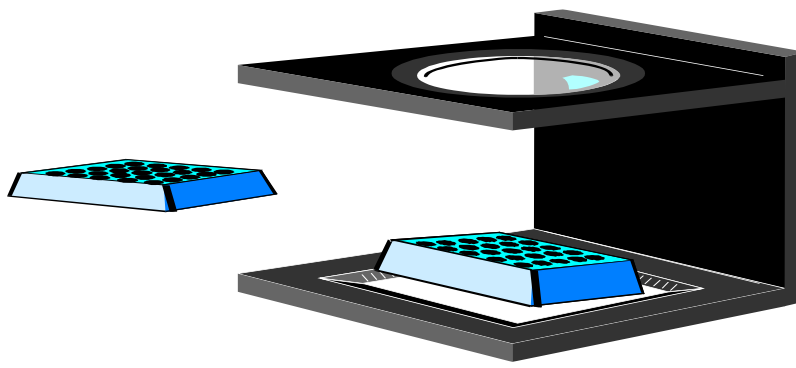
SENSITITRE[®] YeastOne

Microbroth dilution MIC test

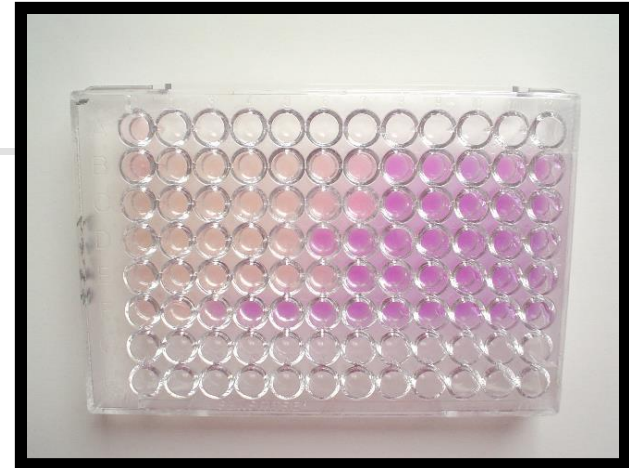
<p>AmB 0.008-16</p> <p>Flu 0.125-256</p> <p>Itra 0.008-16</p> <p>Keto 0.008-16</p> <p>5Fc 0.03-64</p> <p><i>C. albicans</i></p>		<p>MIC</p> <p>0.125</p> <p>1.0</p> <p>0.125</p> <p>0.125</p> <p>0.18</p>
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AccuMed International Ltd [Dutec Diagnostics in Australia]

Sensititre



Visual reading



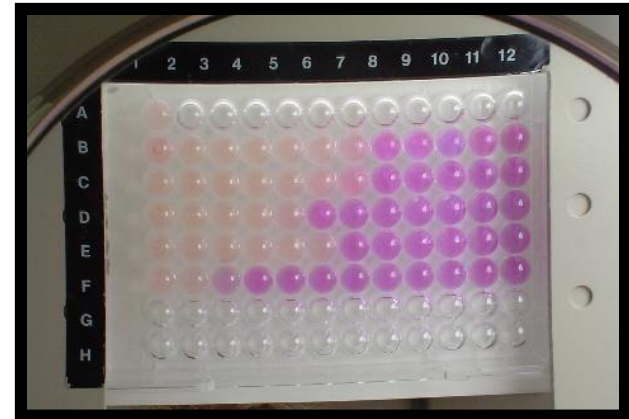
Colorimetric
method: for yeasts

Indicator: alamar-Blue

Endpoint: colour
change to pink

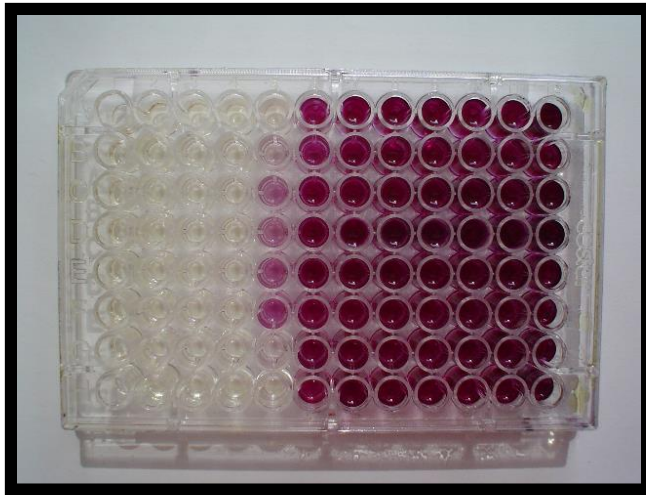


MIC



MTT

3-(4,5-dimethyl-2-thiazil)-2,5-diphenil-2H-tetrazolium-bromide

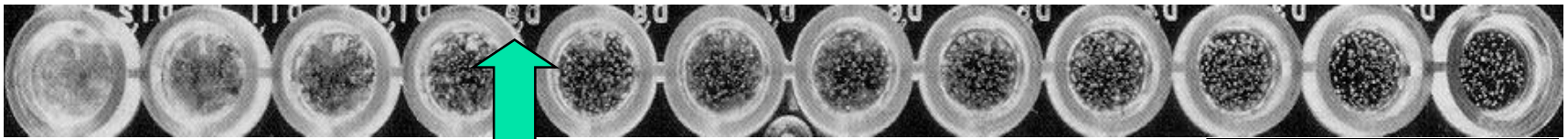


- Colorimetric method: measurement of metabolic activity.
- Yellow tetrazolium enzymatic activity formazan derivate.
- 25 ul RPMI+ 5mg of MTT/ml.
- Incubation 3h.
- After: removal of the content.
- Add 200 ul of isopropanol+ 5% HCl 1M.
- Reading: visual and spectrophotometrically at 540 nm.

Minimal effective concentration: MEC

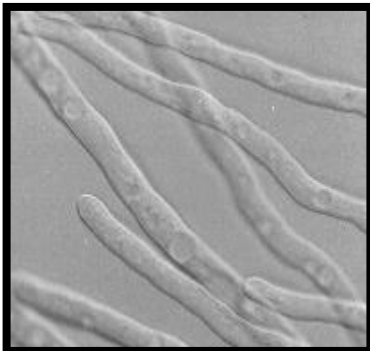
To test equinocandins in molds

MEC



Filament

Cut off
MEC



Aberrant



Fungistatic

Fungitest

Commercial test for 6 antifungal drugs against yeasts.
Inoculum: 1 Mc Farland.

A

B



- Positive: pink.

- Negativo: blue/violet.

- Interpretación: - - S

+ - I

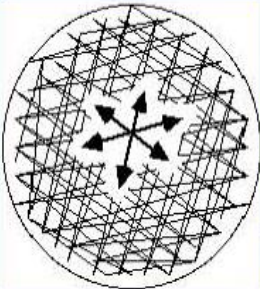
+ + R

- Disdvantage: does not give MIC values.

Diffusion Tests

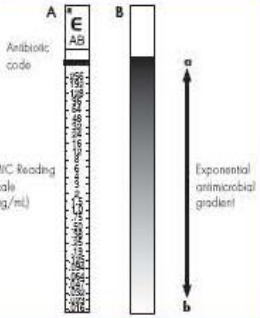
E-test determination and Disk diffusion test

Etest



RPMI1640 +
2% Glucose +
MOPS +
1.5% Agar

0.5-1 McFarland




Antibiotic code

MIC Reading scale (µg/ml)

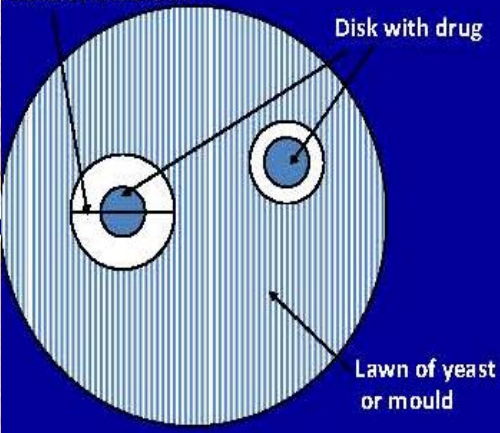
Exponential antimicrobial gradient

35°C for 72h



Measure diameter of zone of inhibition

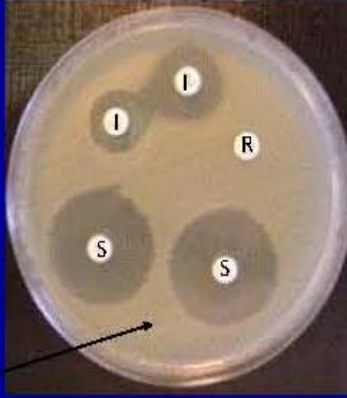
Disk Diffusion Test



Disk with drug

Lawn of yeast or mould

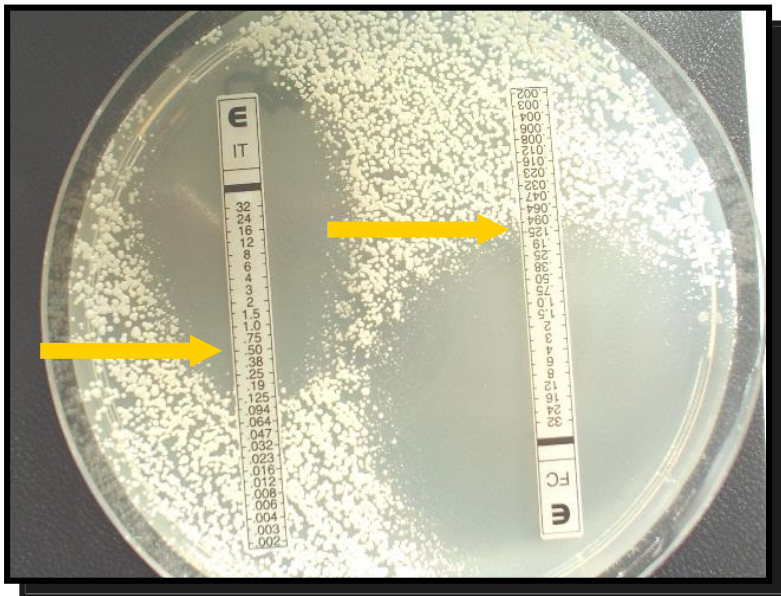
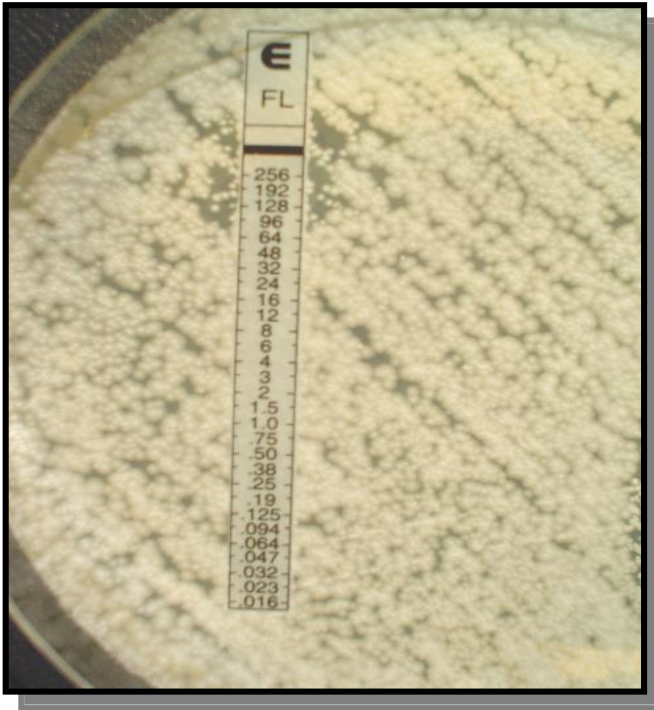
No MIC. Resistant/Intermediate/susceptible based on the diameter of inhibition zone



J. Clin. Microbiol. 2005;43:929-943.

C. krusei: R to FCZ.

C. albicans: MIC 0.96 to 5-FC



C. albicans: MIC 0.25 to ITZ
and 0.19 for 5-FC

Etest Reading Guide for Moulds

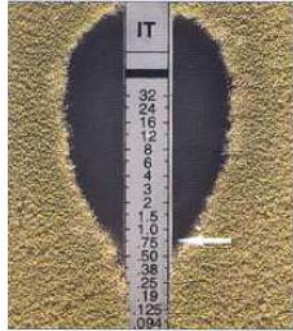


Figure 1. *A. flavus*, read at 72 hours. MIC 0.75 µg/ml.

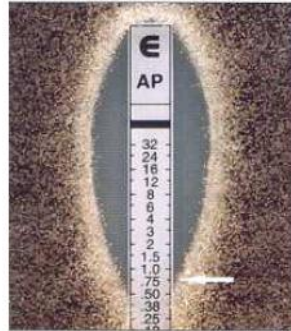


Figure 2. *A. niger*, read at 48 hours. MIC 0.75 µg/ml.

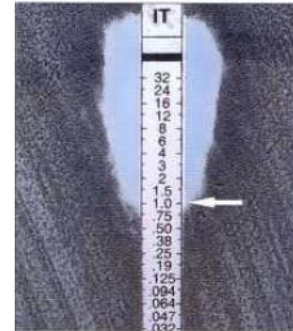


Figure 3. *A. fumigatus*, read at 24 hours. MIC 1 µg/ml.

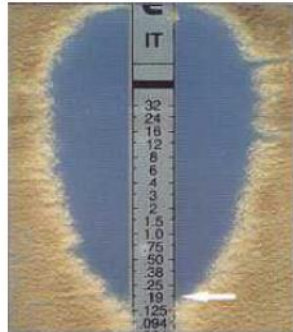


Figure 4. *P. variotii*, read at 48 hours. MIC 0.19 µg/ml.

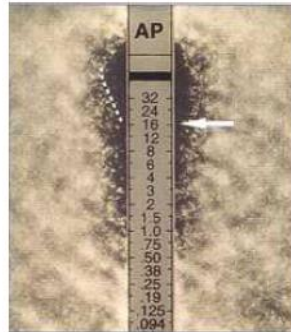


Figure 5. *Fusarium spp.*, read at 24 hours. MIC 16 µg/ml.

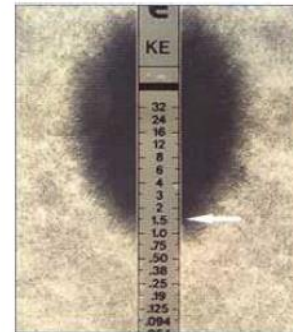


Figure 6. *Rhizopus spp.*, read at 18 hours. MIC 1.5 µg/ml.

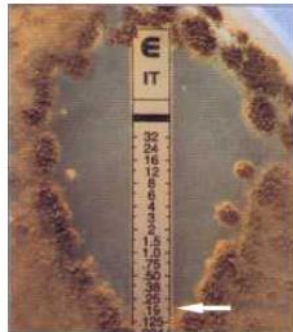


Figure 7. *A. flavus*, read at 24 hours. MIC 0.19 µg/ml.

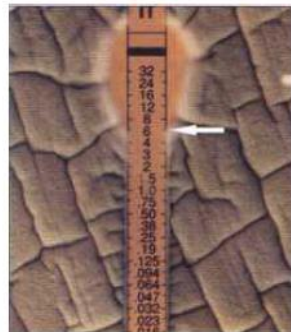


Figure 8. *A. flavus*, read at 24 hours. MIC 6 µg/ml.

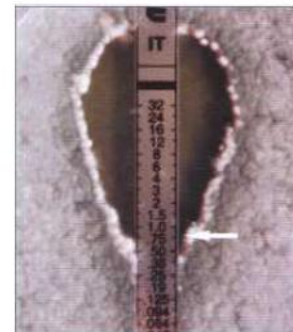


Figure 9. *Fusarium spp.*, read at 24 hours. MIC 0.75 µg/ml.

Conclusion

Methods to determine susceptibility tests

- Microdilution (CLSI- EUCAST)
- Diffusion: E-test and discs
- Colorimetric: MTT, Sensititre
- Commercial kits: Fungitest

